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# A New RP-HPLC Method Development and Validation for Simultaneous Estimation of Sofosbuvir and Velpatasvir in Pharmaceutical Dosage Form

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## ABSTRACT
*A simple, rapid, accurate, precise, specific and sensitive reverse phase-HPLC method has been developed and validated for the simultaneous estimation of Sofosbuvir and Velpatasvir in bulk drug and pharmaceutical dosage form. The chromatographic separation was performed on the Kromasil $C_{18}$ column (250mm×4.6mm, 5µm particle size), using a mobile phase of Buffer: Acetonitrile taken in the ratio 45:55 v/v, at a flow rate of 1.0 ml/min at an ambient temperature of 30˚C with the detection wave length at 260nm. The retention times of Sofosbuvir and Velpatasvir were 2.124 min and 3.334 min respectively. The linearity was performed in the concentration range of 100-600 ppm, 25-150 ppm each of Sofosbuvir and Velpatasvir with a correlation coefficient of 0.999 and 0.999 for Sofosbuvir and Velpatasvir respectively. The percentage purity of Sofosbuvir and Velpatasvir was found to be 98.45% and 99.56% respectively. The proposed method has been validated for specificity, linearity, range, accuracy, precision and robustness were within the acceptance limit according to ICH Q2 (B) guidelines and the developed method can be employed for routine quality control analysis in the bulk and combined pharmaceutical dosage form of Sofosbuvir and Velpatasvir.*

***KEY WORDS:*** ***Sofosbuvir, Velpatasvir, RP-HPLC, Method development, Method Validation.***

## 1.INTRODUCTION
Sofosbuvir (SOF) (Fig.1) is a medication used for the treatment of hepatitis C. It is only recommended with some combination of ribavirin, peginterferon-alfa, simeprevir, ledipasvir, or daclatasvir. Cure rates are 30 to 97% depending on the type of hepatitis C virus involved. Safety during pregnancy is unclear; while, some of the medications used in combination may result in harm to the baby. It is taken orally. The IUPAC name of SOF is Isopropyl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyl-tetrahydrofuran-2-yl] methoxy-phenoxy-phosphoryl] amino] propanoate, Molecular formula $C_{22}H_{29}FN_3O_9P$, Molecular weight 529.4 g/mol. Literature survey indicate that it had been validated by HPLC, and by UV spectrophotometry.

Velpatasvir (VEL) is an NS5A inhibitor which is used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotypes. Velpatasvir (Fig.2) is chemically Methyl {(2S ) - 1 -[ ( 2S, 5S) – 2 - (9 - { 2 -[(2S ,4S) – 1 - {(2R) - 2 -[(methoxycarbonyl) amino] -2 - phenylacetyl} - 4- (methoxymethyl) -2 - pyrrolidinyl] -1 H - imidazol-4-yl} -1, 11 – dihydroisochromeno [4', 3': 6,7] naphtha [1,2-d] imidazol-2-yl) - 5-methyl-1-pyrrolidinyl]-3-methyl-1-oxo-2-butanyl} carbamate used as an anti-cholinergic and anti-spasmodic. Molecular formula $C_{49}H_{54}N_8O_8$, Molecular weight 883.02 g/mol.

The main objective of the present work is to develop a simple, rapid, precise and sensitive reverse phase HPLC method developed and validated for the simultaneous estimation of Sofosbuvir and Velpatasvir in

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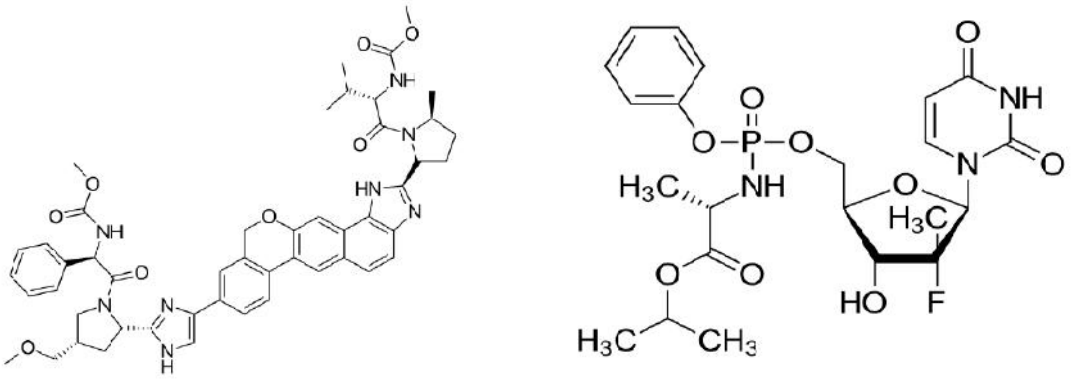
pharmaceutical tablet dosage form. And the method was validated as per ICH Q2 (B) guidelines in terms of specificity, Robustness, Accuracy, Linearity, Limit of detection (LOD), and Limit of Quantification(LOQ).



**a) Velpatasvir** **b) Sofosbuvir**

**Fig.1 The Chemical structure of Velpatasvir and Sofosbuvir**

## 2.MATERIALS AND METHOD

### 2.1 REAGENTS AND CHEMICALS:

The pharmaceutical drug samples Sofosbuvir and Velpatasvir were obtained from Spectrum Pharma Pvt. Ltd., Hyderabad. All the chemicals used of HPLC grade. The pharmaceutical dosage form (EPCLUSA) was purchased from local pharmacy. The solvents used in this work were of HPLC grade and obtained from Merck Specialties Private Limited, Mumbai. Milli Q Water was used in the buffer preparation.

### 2.2 EQUIPMENT:

A Waters e2695 gradient system with Empower-2 software and 2996 module Photo Diode Array detectors equipped with a quaternary solvent delivery pump, automatic sample injector and column thermostat was used for the analysis.

### 2.3 CHROMATOGRAPHIC CONDITIONS:

The column used was Kromasil C18, (250mm×4.6mm, 5µm particle size) for analytical separation. The mobile phase consists of an aqueous solution of 0.1% ortho-phosphoric acid and acetonitrile in the ratio of (45:55%v/v). The flow was adjusted to 1ml/min. The instrument was operated at an ambient temperature. The injection volume was 10µL. The UV detection was achieved at 260 nm which is the isosbestic point shown in Fig.2.

### 2.4 PREPARATION OF ANALYTICAL SOLUTIONS:

#### 2.4.1 PREPARATION OF 0.1%OPA BUFFER SOLUTION:

1ml of Ortho phosphoric acid was pipetted out and dissolved in a 500ml of Milli-Q water taken in a 1000ml Volumetric flask and final volume was made up to the mark with Milli-Q water.

#### 2.4.2 PREPARATION OF MOBILE PHASE:

Mixture of buffer and acetonitrile taken in the ratio 45:55 v/v was degassed in ultrasonic water bath for 10min. Filtered through 0.45µ filtered under vacuum filtration.

#### 2.4.3 DILUENT PREPARATION:

Mixture of Water and acetonitrile taken in the ratio 50:50 v/v was used as diluent.

#### 2.4.4 PREPARATION OF THE SOFOSBUVIR AND VELPATASVIR STANDARD SOLUTION:

Accurately weighed and transferred 40mg of Sofosbuvir and 10mg of Velpatasvir working Standards into a 10ml clean dry volumetric flask, added 3/4$^{th}$ volume of diluent, sonicated for 5 minutes and made up to the final volume with diluent. From the above stock solutions 1ml of Sofosbuvir and 1ml of Velpatasvir were

pipetted into a 10ml volumetric flask and made up to 10ml with diluent to get a mixed standard solution containing concentration of 400ppm and 100ppm of Sofosbuvir and Velpatasvir, so that the drugs Sofosbuvir and Velpatasvir were in the ratio equal to that of the marketed formulation.

**2.4.6 PREPARATION OF SAMPLE SOLUTION:**

Twenty tablets were weighed accurately and finely powdered. A quantity of tablet powder equivalent to 40mg of Sofosbuvir and 10mg of Velpatasvir was accurately weighed and transferred into a 10ml volumetric flask. 5 ml of mobile phase was added to the volumetric flask and ultrasonicated for 25 min; the volume was made up to the mark and mixed well. The solution was filtered through a 0.2µm filter to ensure the absence of particulate matter.

## 3. METHOD DEVELOPMENT AND VALIDATION OF HPLC:

The proposed analytical method was validated according to ICH guidelines (Q2B) with respect to certain parameters such as system suitability, specificity, linearity, accuracy, precision, robustness and ruggedness.

### 3.1 SPECIFICITY:

The specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak[9]. The specificity of the method was determined by observing the interference of any of the possible impurities and excipients.

### 3.2 LINEARITY:

The linearity of the proposed HPLC procedure was evaluated by analysing a series of different concentrations for each of the two analytes and found that the measured peak areas were proportional to concentrations of the analytes. A stock solution of 1000 ppm of two analytes was prepared with diluent. From it, various working standard solutions were prepared in the range of 100 to 600 ppm and 25 to 150 ppm for SOF and VEL respectively and injected into HPLC. It was shown that the selected drugs had linearity in stated range. The calibration plot (peak area versus concentration) was generated by replicate analysis (n=3) at all concentration levels and the linear relationship was evaluated using the least square method.

The retention time of standards was 2.124 min for SOF and 3.334 min for VEL. A typical HPLC chromatogram of the standard mixtures is shown in Fig.5

### 3.3 ACCURACY:

Accuracy study was performed for 50%, 100% and 150 % for SOF and VEL in terms of % recovery. Standard and sample solutions were injected in to HPLC system in triplicate and percentage recoveries of SOF and VEL were calculated. The area of each level was used for calculation of % recovery.

### 3.4 PRECISION:

The precision of the method was ascertained from the peak area obtained by actual determination of six replicates of 400ppm and 100ppm of Sofosbuvir, Velpatasvir respectively. The precision of the assay was also determined in terms of intra- and inter-day variation in the peak areas of a set of drug solutions on three different days. The intra and inter-day variation in the peak area of the drug solution was calculated in terms of relative standard deviation (RSD). The system precision values and method precision values are shown in table no 3&4.

### 3.5 LIMIT OF DETECTION AND QUANTIFICATION:

The limit of detection values for SOF and VEL were 0.15 ppm, and 0.28 ppm, respectively. The limit of quantification values for SOF and VEL were 0.46 ppm, and 0.84 ppm, respectively. The above two parameters are within the range as per the recommendations of USP,2011.

### 3.6 ROBUSTNESS:

Robustness of the developed method was studied by evaluating the influence of small deliberate variations in procedure variables like flow rate (±5%) and change in wave length (±5nm). The robustness was performed for the flow rate variations from 0.8ml/min to 1.2ml/min and the method is robust only in less flow condition and even by change in the mobile phase ±5%. The results are shown in Table 5.

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### 3.7 SYSTEM SUITABILITY:

To ascertain certain system suitability and its effective, the test parameters were checked by repetitively injecting the freshly prepared standard stock solutions at the concentration level 400ppm and 100ppm of Sofosbuvir, Velpatasvir respectively to check the reproducibility of the system.

## 4. RESULTS AND DISCUSSIONS

The present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of SOF and VEL. In order to get the optimized RP-HPLC method, various mobile phases and columns were used. From several trials final method is optimized with the following conditions:

### 4.1 METHOD DEVELOPMENT:

The mobile phase consists of 0.1% ortho-phosphoric acid buffer and acetonitrile in the ratio of 45:55%v/v and the column used was Kromacil C18 column (250mm×4.6mm,5µm particle size). The flow rate was adjusted to 1ml/min. The instrument was operated at an ambient temperature. The UV detection was achieved at 260nm. The injection volume was 10µL.

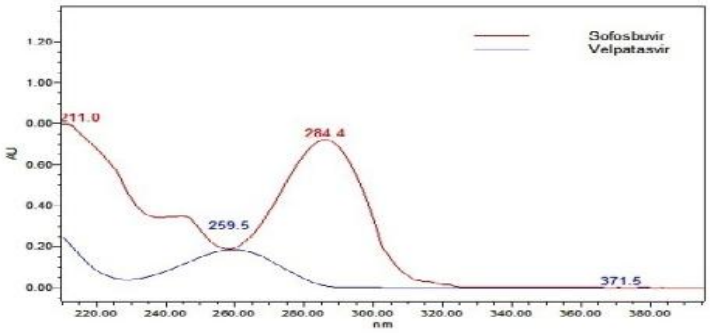


**Fig.2. UV spectra showing Isobestic point of SOF and VEL.**

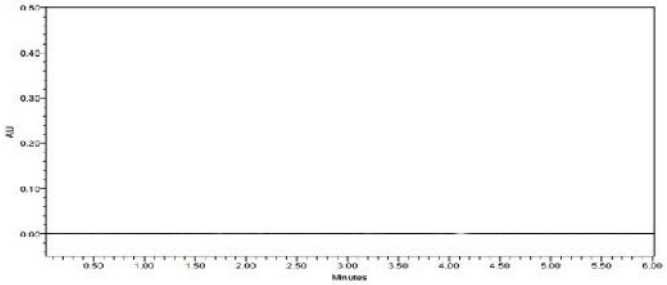


**Fig:3 Blank chromatogram for optimized method**

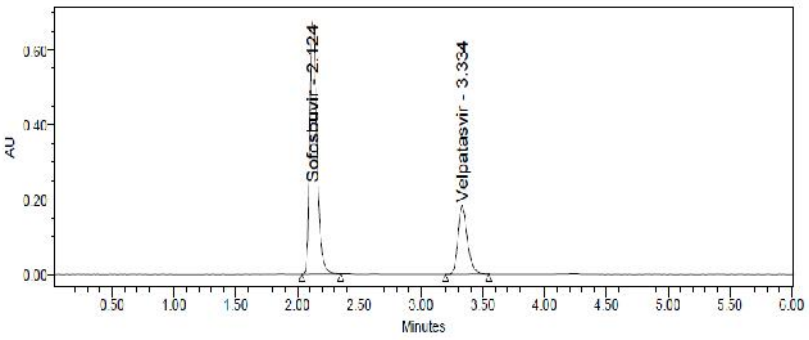


**Fig.4: Standard Chromatogram of Sofosbuvir and Velpatasvir**

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### 4.2 METHOD VALIDATION

Since there is no interference of the other substances in the retention time of the analytical peak. Hence this method was said to be a specific.

The linearity was determined as linearity regression of the claimed analyte concentration of the range 100 to 600 ppm, and 25 ppm to 150 ppm for SOF and VEL respectively. The correlation coefficient was found to be 0.999, and 0.999 for SOF and VEL respectively. Hence the results were obtained with in the limit.

**Table No. 1: Linearity results for SOF and VEL**

| S.NO | Sofosbuvir | | Velpatasvir | |
|---|---|---|---|---|
| | Concentration | Area | Concentration | Area |
| 1 | 100 | 738599 | 25% | 263686 |
| 2 | 200 | 1384340 | 50% | 494926 |
| 3 | 300 | 2044879 | 70% | 735921 |
| 4 | 400 | 2692947 | 100% | 975651 |
| 5 | 500 | 3426881 | 125% | 1239535 |
| 6 | 600 | 4080137 | 150% | 1481491 |
| Correlation Coefficient($r^2$) | 0.999 | | 0.999 | |

**Fig.5. (A) Linearity curve for Sofosbuvir**

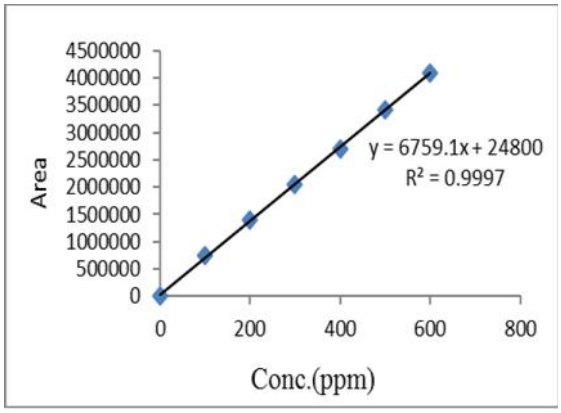
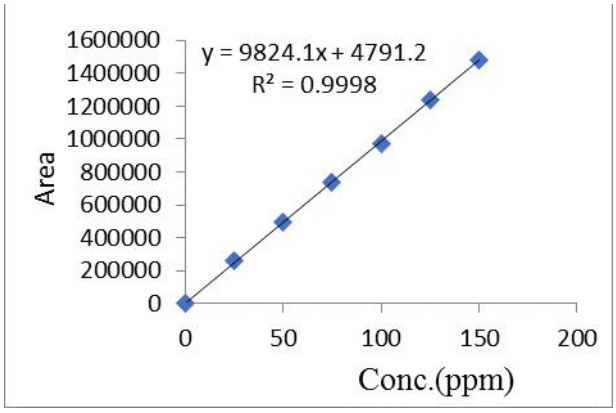



**Fig.5. (B) Linearity curve for Velpatasvir**




### ACCURACY OF THE PROPOSED HPLC METHOD:

Accuracy of the developed method was determined by standard addition method (n=average of 3 analytes). In this method, known amounts of Sofosbuvir and Velpatasvir were supplemented to the previously analysed sample solution and then experimental and true values were compared. Three levels were made corresponding to 50%, 100% and 150% of the nominal analytical concentration. The % recovery was found to be 98.4% for sofosbuvir and 99.5% for Velpatasvir and the results were tabulated in table no.2

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**Table No.2: Accuracy results of SOF and VEL in combined tablet form**

| Drug | Amount taken (ppm) | Amount added* | | Amount recovered* | %Amount found | %Mean |
|---|---|---|---|---|---|---|
| | | % | (ppm) | | | |
| SOF | 400 | 50 | 200 | 196.24 | 98.12 | 98.45 |
| | 400 | 100 | 400 | 393.28 | 98.32 | |
| | 400 | 150 | 600 | 593.43 | 98.91 | |
| VEL | 100 | 50 | 50 | 50.44 | 100.88 | 99.56 |
| | 100 | 100 | 100 | 98.08 | 98.08 | |
| | 100 | 150 | 150 | 149.57 | 99.71 | |

The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The %RSD for method precision was found to be 0.4 and 0.4 for SOF and VEL respectively and the results were tabulated in table no.3 & 4.

**Table No.3: System precision values for SOF and VEL standard solutions**

| S. No | Average area* | | Rt(min)* | |
|---|---|---|---|---|
| | SOF | VEL | SOF | VEL |
| 1 | 2693211 | 966743 | 2.123 | 3.332 |
| 2 | 2686712 | 972077 | 2.123 | 3.333 |
| 3 | 2690798 | 974609 | 2.124 | 3.334 |
| 4 | 2701549 | 971464 | 2.124 | 3.334 |
| 5 | 2686511 | 974506 | 2.124 | 3.334 |
| 6 | 2707195 | 979116 | 2.125 | 3.335 |
| Mean | 2694329 | 973086 | 2.0 | 3.0 |
| SD | 8373.0 | 4112.9 | 0.0 | 0.0 |
| % RSD | 0.3 | 0.4 | 0.0 | 0.0 |

**Table No. 4: Method Precision values for SOF and VEL tablet sample solutions**

| S. No | Average area* | | Rt(min)* | |
|---|---|---|---|---|
| | SOF | VEL | SOF | VEL |
| 1 | 2686663 | 969121 | 2.125 | 3.332 |
| 2 | 2664598 | 961275 | 2.126 | 3.333 |
| 3 | 2675239 | 967314 | 2.126 | 3.334 |
| 4 | 2660349 | 963818 | 2.126 | 3.335 |
| 5 | 2666223 | 965491 | 2.126 | 3.335 |
| 6 | 2671105 | 973645 | 2.127 | 3.335 |
| Mean | 2670696 | 966777 | 2.0 | 3.0 |
| SD | 9380.9 | 4327.4 | 0.0 | 0.0 |
| % RSD | 0.4 | 0.4 | 0.0 | 0.0 |

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The limit of detection values for SOF and VEL were 0.15 ppm, and 0.28 ppm, respectively. The limit of quantification values for SOF and VEL were 0.46 ppm, and 0.84 ppm, respectively. The values were shown in table no.5

**Table No.5 LOD and LOQ values of SOF and VEL**

| Drug name | LOD | LOQ |
|---|---|---|
| **Sofosbuvir** | 0.15 | 0.46 |
| **Velpatasvir** | 0.28 | 0.84 |

The robustness was carried out with minor but deliberate changes in parameters i.e., mobile phase, column temperature, and flow rate as presented in Table 6.

**Table 6: Robustness study of SOF and VEL.**

| Chromatographic Conditions | Average area | | Rt(min) | |
|---|---|---|---|---|
| | SOF | VEL | SOF | VEL |
| Buffer: Acetonitrile 50:50(v/v) | 2760899 | 999143 | 2.12 | 3.18 |
| **Buffer: Acetonitrile 45:55(v/v)** | 2694329 | 973086 | 2.00 | 3.00 |
| Buffer: Acetonitrile 40: 60(v/v) | 2719925 | 1000725 | 2.13 | 3.62 |
| Flow rate (0.8 mL/min) | 3047606 | 1100863 | 2.35 | 3.70 |
| **Flow rate (1.0 mL/min** | 2694329 | 973086 | 2.00 | 3.00 |
| Flow rate (1.2 mL/min) | 2485077 | 900789 | 1.93 | 3.04 |
| Temperature 28°C | 2731323 | 985634 | 2.12 | 3.33 |
| **Temperature 30°C** | 2694329 | 973086 | 2.00 | 3.00 |
| Temperature 32°C | 2755927 | 994687 | 2.12 | 3.33 |

The system suitability parameters like theoretical plates (N), tailing factor (T) were calculated and were found to be more than 2000 and not more than 2 and ascertained that proposed RP-HPLC method was accurate and precise.

| Parameter | Sofosbuvir | Velpatasvir |
|---|---|---|
| **Retention Time** | 2.124 | 3.334 |
| **Theoretical Plates** | 6391 | 8626 |
| **Tailing factor** | 1.34 | 1.21 |

## 5. CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Sofosbuvir and Velpatasvir from pharmaceutical dosage form. The method was validated for parameters like specificity, linearity, accuracy, precision, robustness and system suitability values were found to be within limits. The method was validated as per ICH guidelines. The method has significant advantages, in terms of less retention time, selectivity, and accuracy than previously reported. The validation study indicates that method can be considered suitable for carrying out quality control and routine determination of Sofosbuvir and Velpatasvir in bulk and pharmaceutical dosage form.

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